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**USE OF SPONGOSINE (2-METHOXYADENOSINE) FOR THE
TREATMENT OF PAIN, IN PARTICULAR HYPERALGESIA**

This invention relates to an analgesic and to methods of preventing, treating, or ameliorating pain using the analgesic.

Pain has two components, each involving activation of sensory neurons. The first component is the early or immediate phase when a sensory neuron is stimulated, for instance as the result of heat or pressure on the skin. The second component is the consequence of an increased sensitivity of the sensory mechanisms innervating tissue which has been previously damaged. This second component is referred to as hyperalgesia, and is involved in all forms of chronic pain arising from tissue damage, but not in the early or immediate phase of pain perception.

Thus, hyperalgesia is a condition of heightened pain perception caused by tissue damage. This condition is a natural response of the nervous system apparently designed to encourage protection of the damaged tissue by an injured individual, to give time for tissue repair to occur. There are two known underlying causes of this condition, an increase in sensory neuron activity, and a change in neuronal processing of nociceptive information which occurs in the spinal cord. Hyperalgesia can be debilitating in conditions of chronic inflammation (e.g. rheumatoid arthritis), and when sensory nerve damage has occurred (i.e. neuropathic pain).

Two major classes of analgesics are known: (i) non steroidal anti-inflammatory drugs (NSAIDs) and the related COX-2 inhibitors; and (ii) opiates based on morphine. Analgesics of both classes are effective in controlling normal immediate, or nociceptive pain. However, they are less effective against some types of hyperalgesic pain, such as neuropathic pain. Many medical practitioners are reluctant to prescribe opiates at the high doses required to affect neuropathic pain because of the side effects caused by administration of these compounds, and the possibility that patients may become addicted to them. NSAIDs are much less potent than opiates, so even higher doses of these compounds are required. However, this is undesirable because these compounds cause irritation of the gastro-intestinal tract.

Adenosine A1 receptor agonists are known to act as powerful analgesics (Sawynok, Eur J Pharmacol. (1998) 347, 1-11), and adenosine A2A receptor agonists are known to act as anti-inflammatory agents. However, development of adenosine-based therapies has generally been precluded because they have unacceptable side effects. Selective A1 receptor agonists cause bradycardia, and A2A receptor agonists cause widespread vasodilation with consequent hypotension and tachycardia.

There is, therefore, a need to provide analgesics which are sufficiently potent to control pain perception in neuropathic, inflammatory, and other hyperalgesic syndromes, and which do not have serious side effects or cause patients to become addicted to them.

Spongiosine is a compound that was first isolated from the tropical marine sponge, *Cryptotethia crypta* in 1945 (Bergmann and Feeney, J. Org. Chem. (1951) 16, 981, Ibid (1956) 21, 226). Spongiosine was the first methoxypurine found in nature, and is also known as 2-methoxyadenosine, or 9H-purin-6-amine, 9- α -D-arabinofuranosyl-2-methoxy.

The first biological activities of spongiosine were described by Bartlett *et al.* (J. Med. Chem. (1981) 24, 947-954) who showed that this compound has muscle relaxant, hypothermic, hypotensive, and anti-inflammatory activity in rats (anti-inflammatory activity was assessed by inhibition of carrageenan-induced oedema in a rat paw).

The affinity of spongiosine for the rat adenosine A1 and A2A receptors has been determined. The Kd values obtained were 340nM for the A1 receptor and 1.4 μ M for the A2A receptor (Daly *et al.*, Pharmacol. (1993) 46, 91-100). In the guinea pig, the efficacy of spongiosine was tested in the isolated heart preparation and the EC50 values obtained were 10 μ M and 0.7 μ M for the adenosine A1 and A2A receptors, respectively (Ueeda *et al* J Med Chem (1991) 34, 1334-1339). In the early 1990s the other adenosine receptors (the A2B and A3 receptors) were cloned, but the activity of spongiosine at these receptors was never investigated. The low potency and poor receptor selectivity of this compound led to it being largely ignored as more and more

potent and receptor selective novel compounds were synthesised.

It has surprisingly been found that spongiosine when administered to mammals gives significant pain relief in conditions of increased pain sensitivity (such as neuropathic and inflammatory hyperalgesia), without causing the significant side effects expected from use of purine receptor agonists.

According to the invention there is provided use of spongiosine in the manufacture of a medicament for the prevention, treatment, or amelioration of pain.

The term "spongiosine" is used herein to include spongiosine free base, or a pharmaceutically acceptable salt of spongiosine.

Use of spongiosine according to the invention is particularly concerned with the prevention, treatment, or amelioration of pain other than the early or immediate phase of pain as described above, and is especially concerned with the prevention, treatment, or amelioration of hyperalgesia.

There is also provided according to the invention a method of preventing, treating, or ameliorating pain (in particular hyperalgesia) which comprises administering spongiosine to a subject in need of such prevention, treatment, or amelioration.

Spongiosine has surprisingly been found to be effective in inhibiting pain perception in mammals suffering from neuropathic and inflammatory pain even when administered at doses expected to give concentrations well below those known to activate adenosine receptors. Thus, spongiosine can treat neuropathic and inflammatory pain without causing the significant side effects associated with administration of other adenosine receptor agonists.

No analgesic effect on normal physiological nociception was observed after administration of spongiosine.

Because hyperalgesia is a consequence of tissue damage, either directly to a sensory nerve, or to tissue innervated by a sensory nerve, there are many diseases or conditions in which pain perception includes a component of hyperalgesia.

Spongostin can be used as an anti-hyperalgesic for the prevention, treatment, or amelioration of hyperalgesia caused as a result of neuropathy, including bowel pain, back pain, cancer pain, HIV pain, phantom limb pain, post-operative pain, diabetic neuropathy, polyneuropathy, post-herpes neuralgia, and trigeminal neuralgia.

Other diseases or conditions involving damage to sensory nerves which contain a component of neuropathic pain include, pancreatic pain, pelvic/perineal pain, lower back pain, chest pain, cardiac pain, pelvic pain/PID, joint pain (for example, associated with tendonitis, bursitis, acute arthritis), neck pain, obstetric pain (labour or Caesarean-Section), chronic neuropathic pain, failed back surgery pain, post physical trauma pain (including pain caused by a gunshot wound, a road traffic accident, or a burn), scar tissue pain, acute herpes Zoster pain, acute pancreatitis breakthrough pain (cancer), or for the prevention, treatment, or amelioration of neuropathic or other pain caused by, or associated with, fibromyalgia, myofascial pain syndrome, osteoarthritis, rheumatoid arthritis, sciatica or lumbar radiculopathy, spinal stenosis, temporomandibular joint disorder, renal colic, dysmenorrhoea/endometriosis.

Spongostin can be used as an anti-hyperalgesic for the prevention, treatment, or amelioration of hyperalgesia caused as a result of inflammatory disease, including bowel pain, back pain, cancer pain, fibromyalgia, post-operative pain, osteoarthritis, and rheumatoid arthritis.

Other diseases or conditions in which hyperalgesia plays a prominent role in pain perception because they are associated with chronic inflammation include other arthritic conditions such as rheumatoid spondylitis, gouty arthritis, or asthma, chronic obstructive pulmonary disease, fibrosis, multiple sclerosis, sepsis, septic shock, endotoxic shock, gram negative shock, toxic shock, hemorrhagic shock, adult respiratory distress syndrome, cerebral malaria, organ transplant rejection, pain secondary to cancer, HIV, chronic pulmonary inflammatory disease, silicosis,

pulmonary sarcosis, bone resorption diseases, reperfusion injury, graft v. host rejection, multiple sclerosis, myasthenia gravis, allograft rejections, fever and myalgia due to infection, AIDS related complex (ARC), keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis and pyresis, irritable bowel syndrome, osteoporosis, cerebral malaria, bacterial meningitis, or adverse effects from amphotericin B treatment, interleukin-2 treatment, OKT3 treatment, or GM-CSF treatment.

The pain associated with many of the above diseases or conditions are relatively resistant to NSAIDs and opiates.

It will be appreciated that spongosome may be administered together with a pharmaceutically acceptable carrier, excipient, or diluent.

The appropriate dosage of spongosome will vary with the age, sex, and weight of the subject being treated, and the route of administration.

Preferably spongosome is administered at a dose that gives rise to plasma concentrations one fifth to one thousandth, preferably one fifth to one hundredth, of the minimum plasma concentration of spongosome that gives rise to bradycardia, hypotension or tachycardia side effects in animals of the same species as the subject to which the dose is to be administered.

Alternatively, it is preferred that spongosome is administered at a dose that is one fifth to one fiftieth, preferably one fifth to one tenth, of the minimum dose of spongosome that gives rise to bradycardia, hypotension or tachycardia side effects in animals of the same species as the subject to which the dose is to be administered.

Preferably spongosome is administered at a dose of less than 6mg/kg, and preferably at least 0.01mg/kg, more preferably at least 0.05mg/kg, most preferably at least 0.1mg/kg. More preferably spongosome is administered at a dose of 0.1 to 1mg/kg, or 0.2 to 1mg/kg.

Thus, preferred doses for a 70kg human subject are less than 420mg, preferably at least 0.7mg, more preferably at least 3.5mg, most preferably at least 7mg. More preferably 7 to 70mg, or 14 to 70mg.

Spongosome may be administered by any suitable route, preferably orally, parenterally, sublingually, transdermally, intrathecally, or transmucosally.

Preferably spongosome is administered at a frequency of 2 or 3 times per day.

It has also been found that additive analgesic effects can be obtained if spongosome is administered with another analgesic agent. Thus, spongosome and the other analgesic agent can be administered to obtain a desired level of analgesic effect, each at a lower dose than would be required to achieve that level if either agent was administered alone. Because lower doses of each agent can be administered, side effects associated with administration of higher doses of the agents are reduced. Alternatively, an increased level of analgesic effect can be obtained by administering spongosome and the other analgesic agent at higher doses.

The preferred dosage of spongosome when administered with another analgesic agent is lower than a preferred dosage specified above for administration of spongosome alone.

It is believed that an additive analgesic effect is achieved if the other analgesic agent does not act in the same way as spongosome. Suitable other analgesic agents that may be administered with spongosome include opioid receptor agonists and partial agonists (such as morphine, diamorphine, fentanyl, buprenorphine, codeine, or derivatives thereof), cyclooxygenase inhibitors (such as aspirin, paracetamol, ibuprofen, diclofenac, or derivatives thereof), sodium or calcium channel modulators (such as lignocaine, or gabapentin), or Selective Serotonin Reuptake Inhibitors (SSRI's) (such as paxil).

Example 4 below shows that the anti-hyperalgesic properties of spongosome are unaffected by co-administration of the opioid receptor antagonist naloxone indicating

that spongosome does not act via an opioid receptor. Example 5 below demonstrates the additive analgesic effects of co-administration of spongosome and gabapentin. Gabapentin is effective against neuropathic pain. It is expected that other analgesic agents that are designed to treat neuropathic pain may have additive analgesic effects with spongosome. Such agents include topamax, pregabalin, ziconitide, and cannabinoid derivatives.

Embodiments of the invention are described in the following examples with reference to the accompanying drawings in which:

Figure 1 shows the anti-hyperalgesic actions of spongosome (0.6 mg/kg p.o.) on carrageenan induced hyperalgesia. A: time course (* $p < 0.05$, ** $p < 0.01$ versus vehicle (Sidak's), $p > 0.05$ versus BL over 5 hrs for Spongosome and IND (Dunnett's)); B: dose dependency of the anti-hyperalgesic effect;

Figure 2 shows the anti-hyperalgesic actions of spongosome (0.6 mg/kg p.o.) in the chronic constriction injury model of neuropathic pain (* $p < 0.05$, ** $p < 0.01$ vs veh (ANOVA Sidak's);

Figure 3 shows the effect of spongosome (0.6 mg/kg p.o.) on A: blood pressure in normal rats; B: heart rate;

Figure 4 shows the effect of spongosome (0.6 mg/kg p.o.) in the presence and absence of naloxone in the chronic constriction injury model of neuropathic pain; and

Figure 5 shows the additive effect of spongosome and gabapentin in the chronic constriction injury model of neuropathic pain.

Examples

Example 1

Figure 1: A. Spongosome (0.624mg/kg p.o.) inhibits carrageenan (CGN) induced thermal hyperalgesia (CITH) with comparable efficacy to indomethacin (3mg/kg, po). B. Concentration-response relationship for Spongosome at 3 hrs post dosing. Carrageenan (2%, 10 microlitres) was administered into the right hind paw. A heat source was placed close to the treated and untreated hind paws, and the difference in

the paw withdrawal latencies is shown. Spongostin was administered at the same time as carrageenan.

Example 2

Figure 2: Spongostin (0.624mg/kg p.o.) inhibits thermal hyperalgesia caused by chronic constriction injury of the rat sciatic nerve. Under anaesthesia the sciatic nerve was displayed in the right leg, and four loose ligatures tied round the nerve bundle. After approximately two weeks the rats developed thermal hyperalgesia in the operated leg as judged by the difference in paw withdrawal latencies of the right and left paws. Administration of spongostin reduced the hyperalgesia as shown by the reduction in the difference between the withdrawal latencies. Spongostin was as, or more, effective than carbamazepine (CBZ, 100mg/kg s.c.)

Example 3

Figure 3: Spongostin (0.624 mg/kg p.o.) has no significant effect on blood pressure or heart rate. An implantable radiotelemetry device was placed in the abdominal cavity of 6 rats per group. The pressure catheter of the device was inserted in the abdominal aorta and two electrodes tunnelled under the skin in a lead II position (left side of abdominal cavity/right shoulder). Individual rats were placed in their own cage on a radioreceptor (DSI) for data acquisition. A: blood pressure, B; heart rate.

Example 4

Figure 4: Spongostin (1.2 mg/kg p.o.) inhibits static allodynia caused by chronic constriction injury of the rat sciatic nerve, both in the presence and absence of naloxone (1 mg/kg s.c.). Under anaesthesia the sciatic nerve was displayed in the right leg, and four loose ligatures tied round the nerve bundle. After approximately two weeks the rats developed static allodynia in the operated leg as judged by the difference in paw withdrawal thresholds of the right and left paws. Administration of spongostin reduced the hyperalgesia as shown by the increased paw withdrawal threshold (PWT) in the presence and absence of naloxone. Veh: vehicle.

Example 5

Figure 5: Spongostin and gabapentin inhibit static allodynia caused by chronic constriction injury of the rat sciatic nerve. Spongostin and gabapentin were administered (p.o.) in different proportions as indicated in the drawing. The total dose administered is shown on the horizontal axis, and the paw withdrawal threshold (PWT) on the vertical axis. The predicted anti-hyperalgesic effect (derived from the dose response curves obtained with each agent alone) if the effects of the two compounds are additive is shown (●). The observed effects are indicated by (■). It is apparent that the observed effects are not significantly different from those predicted by additivity.

Spongostin is effective in inhibiting pain perception in mammals suffering from neuropathic and inflammatory pain even when administered at doses expected to give concentrations well below those known to activate adenosine receptors. At these doses it can be seen that neither the heart A1 receptors nor the vascular A2A receptors are sufficiently stimulated to cause a change in the cardiovascular status of the animals.

Spongostin can therefore be used as an anti-hyperalgesic which can be administered orally for the treatment of hyperalgesia caused as a result of neuropathy or inflammatory disease, including bowel pain, back pain, cancer pain, fibromyalgia, HIV pain, phantom limb pain, osteoarthritis, rheumatoid arthritis, post-herpes neuralgia, trigeminal neuralgia, polyneuropathy, diabetic neuropathy and post-operative pain.